UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLUTION PREVENTION/OFFICE OF PESTICIDE PROGRAMS

MEMORANDUM

DATE: 13 December 2011

SUBJECT: Science Review in Support of the Registration of Natamycin TGAI, a Technical Grade Active Ingredient (TGAI) Product, Containing 91.02% Natamycin, a New Active Ingredient. Prenatal Development Rationale.

Decision Numbers:	434160 & 434161
DP Numbers:	378661 & 378664
EPA File Symbol Numbers:	87485-R
Chemical Class:	Biochemical
PC Code:	051102
CAS Number:	7661-93-8
Tolerance Exemptions:	Pending
MRID Numbers:	48613501

FROM: Russell S. Jones, Ph.D., Senior Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

TO: Cheryl Greene, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511P)

ACTION REQUESTED

On behalf of DSM Food Specialties B.V. (DSM) and in response to a request for additional information (see Memorandum from R. S. Jones to C. Greene, dated 04/04/2010), A. Jovanovich (Keller & Heckman, LLP) submitted a rationale with supporting information and data from the open technical literature in fulfillment of the Prenatal Developmental Toxicity Guideline Data Requirement (OCSPP 870.3700). The submitted information is reviewed below for its adequacy in supporting the Prenatal Developmental Toxicity Guideline Data Requirement.

RECOMMENDATIONS AND CONCLUSIONS

1. The rationale and supporting information submitted by the registrant in fulfillment of the Prenatal Developmental Toxicity Guideline Data Requirement (OCSPP 870.3700) are **ACCEPTABLE**

2. Based on the data and the weight of evidence, fetal exposure from oral ingestion of natamycin in/on treated mushrooms by the mother would likely be extremely low. There are no concerns for subchronic, chronic, and reproductive/developmental toxicity resulting from dietary exposure to natamycin-treated mushrooms.

EXECUTIVE SUMMARY

Natamycin is not a subchronic toxicant in rats up when administered in the diet at up to 45 mg/kg bw/day for 96 days, nor in dogs at up to 12 mg/kg bw/day for 3 months (Hutchinson et al. 1966; Levinskas et. al. 1966; Van Ecken et. al. 1984) . Natamycin is not a carcinogen in rats or dogs administered natamycin in the daily diet for up to two years, based on a lack of observable differences in tumors relative to untreated controls (Levinskas et. al. 1963 & 1966). The NOAEL for chronic toxicity was 22.4 mg/kg bw/day in rat and 6.25 mg/kg bw/day in dog, based on reduced body weight.

Natamycin is not a reproductive or developmental toxicant when administered to experimental animals at $\geq$ 50 mg/kg bw/day in three-generation and two-generation studies with rats (Cox et. al. 1973).

Exposure to dietary natamycin is expected to be extremely low. Dietary natamycin is rapidly metabolized by stomach acids, poorly absorbed by mammalian systems; natamycin and its degradates are rapidly excreted in the feces within 24 hrs when orally ingested (Blankwater and Hespe, 1979; Hespe and Meier, 1980; Morgenstern and Muskens, 1976).

Natamycin is a high molecular weight compound (666 Daltons) with low solubility in water (30-50 ppm at 20-25 °C) and many organic solvents. Chemical compounds having molecular weights >600 Daltons are not known to diffuse across the placental barrier of humans (Pacifici and Nottoli, 1995); there are no known active transport mechanisms for natamycin.

RISK ASSESSMENT

In a previous review of the data (see Memorandum from R. S. Jones to C. Greene, dated 04/11/2011), it was observed that in field crop field trials, maximum residues on button mushrooms following application of Natamycin at the maximum label use rate results in residues up to 0.2370 mg a.i./kg in unwashed mushrooms or up to 0.0755 mg a.i./kg in washed mushrooms.

Based on per capita consumption of all mushroom commodities in the United States (USDA/ERS, 2010), dietary intake from treated, unwashed mushrooms is conservatively estimated to be no more than 0.00030 mg a.i./kg bw/person/day (see Memorandum from R. S. Jones to C. Greene, dated 04/11/2011). This value is well below any known acute oral, subchronic and chronic dietary, reproductive, and developmental endpoints for Natamycin by many orders of magnitude (see Executive Summary above). In addition, the estimated dietary intake from unwashed, treated mushrooms also is well below the Acceptable Dietary Intake (ADI) of 0.3 established by Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2001& 2006) and an ADI of 0.1 established European Food Safety Authority (EFSA, 2009).

Based on the data and the weight of evidence, fetal exposure from oral ingestion of natamycin by the mother would likely be extremely low. There are no concerns for subchronic, chronic, and reproductive/developmental toxicity resulting from dietary exposure to natamycin-treated mushrooms.

STUDY SUMMARIES

Overview of the Active Ingredient

Natamycin is a polyene macrolide antimycotic biochemical compound (i.e. an anti-fungal agent) originally derived from the common soil microorganism, *Streptomyces natalensis* (see Figure 1).

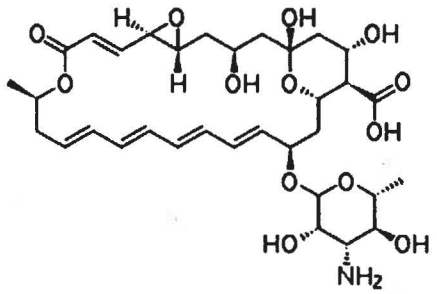

Figure 1. Natamycin

Natamycin TGAI (EPA File Symbol No. 87485-R; containing 91.02% Natamycin as its active ingredient)

Natamycin L (EPA File Symbol No. 874856-E; containing 10.34% Natamycin as its active ingredient) is an end use product to be used to prevent dry bubble disease (*Verticillium fungicola*) on commercially-produced button mushrooms (*Agaricus bisporus*). Natamycin L is added to the irrigation water in enclosed mushroom production facilities.

Formulated Product Name:	Natamycin L
Active Ingredient (a.i.):	Natamycin
IUPAC Chemical Name:	(1*R*,3*S*,5*R*,7*R*,8*E*,12*R*,14*E*,16*E*,18*E*,20*E*,22*R*,24*S*,25*R*,26*S*)-22-[(3-amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,26-trihydroxy-12-methyl-10-oxo-6,11,28-trioxatricyclo[22.3.1.$0^{5,7}$]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid
CAS Index Name for a.i.:	6,11,28-trioxatricyclo[22.3.1.$0^{5,7}$]octacosa-8,14,16,18,20-pentaene-25-carboxylic acid, 22-[(3-amino-3,6-dideoxy-β-D-mannopyranosyl)oxy]-1,3,26-trihydroxy-12-methyl-10-oxo-, (1R,3S,5R,7R,8E,12R,14E,16E,18E,20E,22R,24S,25R,26S)-
CAS Registry No. for a.i.:	7661-93-8
Other a.i. Common Names:	Pimaricin Tennectin Delvocid
Molecular Formula of a.i.:	$C_{33}H_{47}NO_{13}$
Molecular Weight of a.i.:	665.725 g/mol
Nominal conc. a.i. (TGAI):	91.02% w/w
Nominal conc. a.i. (EP):	10.13% w/w (as listed in MRID 48105406; (see Note to RAL below).

Prenatal Developmental Toxicity (OCSPP 870.3700; OECD 414; MRID 48613501):

No new studies were submitted. In lieu of a Guideline study, the registrant developed a rationale

supported with information and data obtained from the open technical literature. The rationale relies primarily on a comprehensive review of Natamycin by the European Food Safety Authority (EFSA, 2009) and its associated citations. The EFSA review relies on The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviews on natamycin (aka pimaricin) of 1968, 1976, and 2002. The EFSA/JECFA reviews rely on three subchronic toxicity studies: and two in the rat (Hutchinson et al. 1966; Levinskas et. al. 1966) and one in the dog (Van Ecken et. al., 1984); two 2-year chronic studies, respectively in the rat (Levinskas et. al., 1963 & 1966) and in the dog (Levinskas et. al., 1966); a three-generation reproductive toxicity study in the rat (Cox et. al. 1973); and a three-generation developmental toxicity study in the rat (Cox et. al., 1973). A rabbit developmental study was also considered by JECFA, but this study was independently reviewed by the Agency (see Memorandum from R. S. Jones to C. Greene, dated 04/04/2010; and MRID 48105512) and deemed unacceptable. In addition, human data were considered by EFSA (2009), but these studies were not cited in this document as they had not been reviewed by the Human Studies Review Board.

The EFSA (2009) review also provided data in regard to the low potential for absorption and rapid excretion of natamycin and its degradates from mammalian systems.

Summaries of Reproductive and Developmental Toxicity Studies Reviewed by EFSA (2009)

~~Three subchronic toxicity studies, two 2-year chronic studies, three-generation reproductive~~ toxicity study in the rat a three-generation developmental toxicity study in the rat evaluated by EFSA (2009) are summarized below:

A. Subchronic Toxicity Studies

1. Hutchinson et al. 1966: 30 rats (15 male, 15 female) were administered natamycin in the diet (45 mg/kg bw/day) for up to 96 days. There were no significant differences in hematology, organ histology, and mean body weight gain between treatment and control groups. The NOAEL was >45 mg/kg bw/day in this study.

2. Levinskas et. al. 1966: 200 rats (20 male, 20 female per dose group) were administered natamycin in the diet (0, 10, 45, 190, and 750 mg/kg bw/day) for up to 96 days. There were no significant differences in hematology and organ histology between treatment and control groups. Food consumption declined in the highest dose group (23% and 17%, respectively, in males and females) compared with controls. In the 190 mg/kg bw/day group, mean food consumption declined 5% for both sexes and mean body weight was 85% of the mean body weight of untreated controls. The NOAEL was 45 mg/kg bw/day in this study, based on reduced body weight.

3. Van Ecken et. al. 1984: 12 Beagle dogs (2 male, 2 female per dose group) were

administered natamycin in the diet (0, 12, and 25 mg/kg bw/day) for 3 months. Transient diarrhea was observed in one male (for 39 days) and 2 females (8 or 10 days) in the high dose group, together with a slight decline in body weight for all members of the high dose group. The transient diarrhea was not deemed to be treatment related. The NOAEL was 12 mg/kg bw/day in this study, based on reduced body weight.

Conclusion: Natamycin is not a subchronic toxicant in rats up when administered in the diet at up to 45 mg/kg bw/day, nor in dogs at up to 12 mg/kg bw/day.

B. Chronic Toxicity and Carcinogenicity

1. Levinskas et. al. 1963 & 1966: 350 rats (35 male and 35 female per dose group) were administered natamycin in the diet (males: 0, 4.5, 11, 22.4, and 46.3 mg/kg bw/day; females: 0, 7.58, 15.4, 30.4, and 63.7 mg/kg bw/day) for two years. All rats survived the duration of the study. Decreased food intake and growth were observed only I the highest dose group. After 6 months, females in the 30.4 mg/kg bw/day group had a significant increase in hemoglobin value, but hemoglobin values in the lower and higher dose groups were unaffected and, therefore, not considered to be treatment related. Hematocrit values did not differ significantly from controls. Mammary gland adenocarcinoma, pituitary, chromophobe adenomal, and uterine/vaginal polyps were observed in all animals but the numbers were not significantly different between treatment and control animals. The NOAEL was 22.4 mg/kg bw/day in this study, based on reduced body weight.

2. Levinskas et. al. 1966: 24 Beagle dogs were administered natamycin in the diet at 0, 125, 250, and 500 mg/kg for two years. Body weights of all animals increased through the 15th month of the study. The daily diet was then reduced by approximately 17% due to excessive obesity in dogs at the highest dose level. The dietary reduction resulted in a substantial weight reduction in all dogs. Dietary levels in the 250 mg/kg dose group did not affect body weight gain or maintenance. There were no observed changes in hematology or clinical chemistry values in any dose group relative to controls that were ascribed to natamycin. Males in the 125 and 250 mg/kg dose group had reduced mean liver weights relative to controls, but there were no significant differences in liver weights at the higher dose levels, indicating that the effects on liver weights were not treatment related. The NOAEL was 250 mg/kg diet (6.25 mg/kg bw/day) in this study, based on reduced body weight.

Conclusion: Natamycin is not a carcinogen. The NOAEL for chronic toxicity was 22.4 mg/kg bw/day in rat and 6.25 mg/kg bw/day, based on reduced body weight.

Reproductive and Developmental Toxicity

1. Levinskas et. al. 1963 & 1966: An unknown number of rats were administered 0 or 1000 mg/kg of natamycin in the diet for an unknown period of time. There were no significant differences in fertility, gestation, lactation, and viability between treatment and control animals. A low incidence of unspecified abnormalities was reported among pups in the study were they were deemed to be unrelated to natamycin treatment. Information provided by this study summary is deemed to be too limited to be of use in a risk assessment.

2. Cox et. al. 1973: In a three-generation reproductive study, 150 rats (10 males, 20 female per dose group) were administered natamycin in the diet at 0, 5, 15, 50, or 100 mg/kg bw/day for 11 weeks. Animals in the highest dose group had an increased number of fetuses born dead, and a decrease in animals born alive surviving 21 days in the F1 generation. There were depressed pup weights observed in the second generation. There were no effects on growth, reproduction, or gross microscopy pathology. Fertility, gestation, viability and lactation indices were within normal limits for both litters of all three generations. The NOAEL was 50 mg/kg bw/day in this study, based on growth and reproduction.

3. Cox et. al. 1973: A developmental toxicity study was conducted on the second litter of rats from the F1 generation (from the study described paragraph 2 above). They were reared to maturity and mated with untreated control males. The females (20) were administered natamycin in the diet at 0, 5, 15, or 50 mg/kg bw/day by intragastric intubation on Days 6 and 18 of gestation then euthanized and examined on Day 20. No effects on nidation or maternal or fetal survival were observed. Soft tissue abnormalities were no different than those observed in untreated controls. The NOAEL was >50 mg/kg bw/day in this study, based on growth and reproduction.

Conclusion: Based on the weight of evidence, the NOAEL for reproductive and developmental toxicity is ≥50 mg/kg bw/day.

Absorption, Distribution, Metabolism, and Excretion

1. Blankwater and Hespe, 1979: Radiolabeled ^{14}C-natamycin was orally administered in a single dose (50 mg/kg bw) to 5 Wistar rats, and radioactivity was monitored by whole-body autoradiography. After 1 hour, radioactivity was observed only in the esophagus, stomach, and small intestine, with some located in the caecum at 2 hours post dosing. At 4 hours postdosing, the radioactivity was primarily in the colon and at 24 hours was substantially reduced throughout the GI tract. The majority of the radioactivity was eliminated in the feces within 24 hours, with small traces of radioactivity in the liver, kidneys, and fatty tissue, indication very low absorption of dietary natamycin and rapid

excretion in the feces.

In the bioautographic portion of the study, 4 female rats were orally administered a single dose (50 mg/kg bw) of non-radiolabeled natamycin. Antibiotic activity was measured in whole body sections of euthanized animals at 1, 2, 4, 8, and 24 hrs post treatment via exposure to agar plates inoculated with *Sacharromyces cereviseae*. Antibiotic activity was restricted primarily to the gastrointestinal tract and activity ceased in <24 hrs. No antibiotic activity was observed in colon samples.

According to the study authors, lack of antibiotic activity and the presence of radioactivity in the caecum and colon 24 hrs post-dosing indicate the breakdown of natamycin into antibiotic-inactive compounds by bacteria in the caecum and colon.

2. Hespe and Meier, 1980: In an autoradiography study, four female Beagle dogs were administered radiolabeled natamycin a plastic capsule, on cheese, in gelatin capsules, and in a 1% starch solution. Regardless of the method of administration, the majority of radioactivity was detected in the feces with small amounts (<4%) observed in the urine within 24 hrs of dosing. The small amount of radioactivity in the urine is indicative of low absorption of dietary natamycin. Following intravenous administration of Radiolabeled natamycin to dogs, radioactivity was excreted primarily in the bile. Based on a comparison of dietary and intravenous administration data, the authors concluded that no more that 5% of natamycin is absorbed.

3. Morgenstern and Muskens, 1976: When natamycin is mixed with gastric juice, approximately 50% is broken down within 1 hour. When fed to rats, radiolabeled natamycin, losses from the stomach are 33-34% and 0-31% respectively, in fasted and non-fasted rats.

Conclusions: Natamycin is poorly absorbed from dietary sources and is rapidly broken down and excreted from mammal systems, primarily in the feces with 24 hours. Developmental and reproductive exposure is further limited by the size (666 Daltons) and relative insolubility (30-50 ppm in water) of natamycin. Molecules with molecular weights >600 Daltons do not diffuse across the placental barrier of humans. There are no known active transport mechanisms for natamycin.

Overall Conclusions

The EFSA (2009) review concluded that Natamycin is poorly absorbed by mammals and that it does not pose any dietary concerns based on current usage. BPPD concurs with this conclusion.

REFERENCES

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cc: Russell S. Jones, Ph.D., Sr. Biologist, BPPD/BPB, Cheryl Greene, RAL, BPPD Chron File, IHAD/ARS
R. S. Jones, FT, PY-S: 12/13/2011.